A microfluidic cell culture device with integrated microelectrodes for barrier studies.

Tan, Hsih-Yin; Dufva, Martin; Kutter, Jörg P.; Andresen, Thomas Lars

Publication date:
2016

Document Version
Peer reviewed version

A microfluidic cell culture device with integrated microelectrodes for barrier studies

Hsih-Yin Tan[a,b], Martin Dufva[a], Jörg P. Kutter[c], Thomas Lars Andresen[a,b,1]

[a] Technical University of Denmark, Department of Micro and Nanotechnology, Ørsteds Plads, 2800 Kgs. Lyngby, Denmark
[b] Technical University of Denmark, Center for Nanomedicine and Theranostics, Ørsteds Plads, 2800 Kgs. Lyngby, Denmark
[c] Department of Pharmacy, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark

ABSTRACT
We present an eight cell culture microfluidic device fabricated using thiol-ene 'click' chemistry with embedded microelectrodes for evaluating barrier properties of human intestinal epithelial cells. The capability of the microelectrodes for trans-epithelial electrical resistance (TEER) measurements was demonstrated by using confluent human colorectal epithelial cells (Caco-2) and rat fibroblast (CT 26) cells cultured in the microfluidic device.

INTRODUCTION
Trans-epithelial electrical resistance (TEER) is one of the widely used and conceivably the most straightforward technique for understanding the integrity of an epithelial or endothelial cell layer (1, 2). This paper reports on a straight-forward method of embedding microelectrodes in a multi-layer thiolene microfluidic chip for measuring TEER across cell layers. The reported microelectrodes embedded in the microfluidic device were fabricated using two different metals and are in direct contact with the reagents within the microchannels. The capability of the microelectrodes for TEER measurements was demonstrated by using confluent human colorectal epithelial cells (Caco-2) and rat fibroblast (CT 26) cells cultured in the microfluidic device. Capability of the microelectrodes in sensing dynamic changes of the barrier integrity was demonstrated by challenging the Caco-2 monolayers with a membrane enhancer that temporary disrupted the cell barrier property. Immunofluorescence staining towards the tight junctional protein, ZO-1, and nucleus were also conducted to observe the morphology of the cells. Such a microfluidic cell culture platform, open new opportunities for real-time monitoring of the cell barrier properties.

METHODS AND MATERIALS
EMBEDDING THE MICROELECTRODES ONTO THE MICROCHIP
The microfluidic device was fabricated using thiol-ene 'click' chemistry. The design and fabrication of the microfluidic chip have been reported earlier (3). Once the top and bottom fluidic layers were cured by UV exposure and demolded from the PDMS molds, microelectrode grooves were micromilled to the bottom layer (Fig. 1b). Briefly, the top and bottom fluidic layers were fabricated by mixing pentaerythritol tetrakis-(3-mercaptopropionate) (tetra-thiols moieties) with tri-allyl-tri-azine (tri-allyl moieties) in stoichiometric ratios. The mixture was poured onto the PDMS molds, exposed to UV for 40s and de-molded. These layers were used as the upper and lower fluidic layers of the microfluidic chip. Grooves for the microelectrodes (present only in the lower fluidic layer of the microchip) were micro-milled into the cured thiol-ene piece (Fig. 2a). As for the porous membrane, the surface of the membrane was modified by coating a mixture of tri-allyl-tri-azine (tri-allyl moieties) and trimethylopropane tris-(2-mercaptopropionate) (tri-thiol moieties). Before bonding the fluidic layers and modified Teflon membrane, holes for the inlets, outlets and electrode ports were drilled into the partially cured thiol-ene layers (Fig. 1b). The different pieces of cured thiol-ene layers were aligned and placed on each other. A slight pressure was applied onto the pieces to ensure good contact between the pieces. The entire microchip was further exposed to UV radiation for another 1min on each side of the microchip. The bottom electrodes were fabricated using an indium alloy, InBiSn, which has a melting temperature of ≈ 62 °C. Therefore, to fabricate the bottom electrodes, the sealed microchip was first placed onto a hotplate set at 80 °C and pieces of the InBiSn metal, with length of 5 mm, were inserted into the electrode ports (connecting to the lower fluidic layer) on the thiol-ene microchip. Heat from the hotplate and the microchip resulted in InBiSn

metal melting. Slight pressure was applied manually to push the melted metal into the electrode grooves. Once the electrodes were formed, electric wires of diameter 0.4 mm were inserted to the liquid metal. These wires would act as the connecting wires to the multimeter. Once the microchip was cooled down in room temperature, the InBiSn electrode solidified, retaining the shape (Fig. 1e). The size of electrode for measuring TEER, was about about 0.6–0.8 mm in diameter and thickness of 0.3 mm (Fig. 1e).

The top electrodes were fabricated by fitting two pieces of platinum (Pt) wires (diameter of 0.5 mm, length of 5 mm) into the electrode ports drilled through the top fluidic layer. Two connecting Cu wires were soldered to the Pt wires (Fig. 1c). To fix the electrodes in position, UV-epoxy (NOA81; Norland, USA) was applied at the junctions between connecting wires and thiol-ene chip. The entire chip was exposed to UV light for 30s.

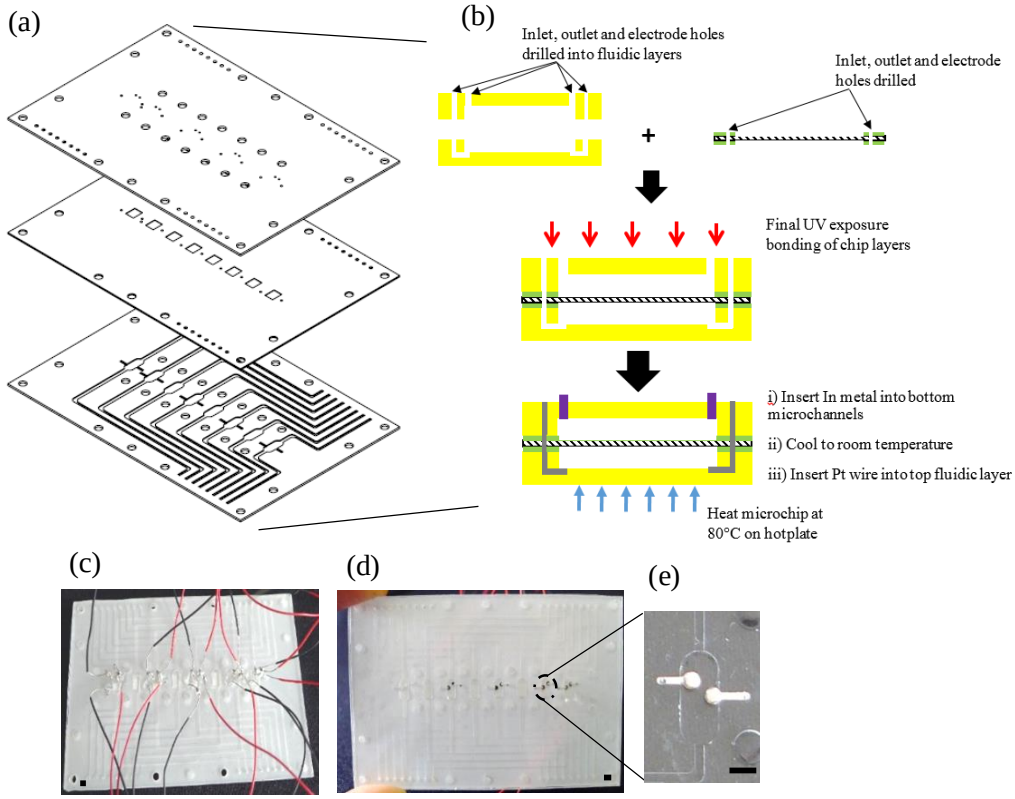

Figure 1. Design and development overview of microelectrodes embedded in the multi-layer and multi chamber thiol-ene microchip for Caco-2 cell culture: (a) Schematic drawing of the 3 layers present in the thiol-ene microchip. (b) Schematic drawing of the process to embed the microelectrodes on the microchip. (c) Top view of the completed microchip with embedded electrodes and connecting wires. (d) Underside of thiol-ene microfluidic chip with the InBiSn electrode embedded in the microchamber. (e) Expanded view of the InBiSn electrode. (Scale bar = 2mm).

RESULTS AND DISCUSSION

To investigate the working feasibility of the microelectrodes for TEER measurements, two different cell lines were experimented i) Caco-2 cells (human colorectal epithelial cells) which are known to form tight junctions and ii) rat fibroblast (CT 26) cells, which are incapable of forming tight junctions. The TEER measured for Caco-2 cells was ≈ 861 $\Omega \cdot cm^2$ and for CT 26, the TEER measured was ≈ 56 $\Omega \cdot cm^2$ (Fig 2).

Further demonstrations of the capability of the microelectrodes in sensing dynamic changes of the barrier integrity of the cell monolayers were presented with the introduction of two different concentrations of membrane enhancer (tetradecyl-β-D-Maltoside (TDM)) to the cell monolayers. Challenging the cells cultured in the microfluidic device with 100 µM and 400 µM of TDM, resulted in percentage decrease in TEER values of ≈ 60.4 % and ≈ 20.9 %, respectively, of initial values (Fig. 3A). To further analyse the morphology of the barrier integrity of the Caco-2 cells, immunostaining of the tight-junctional protein, ZO-1, and nucleus of the Caco-2 cells were conducted (Fig 3B-D).

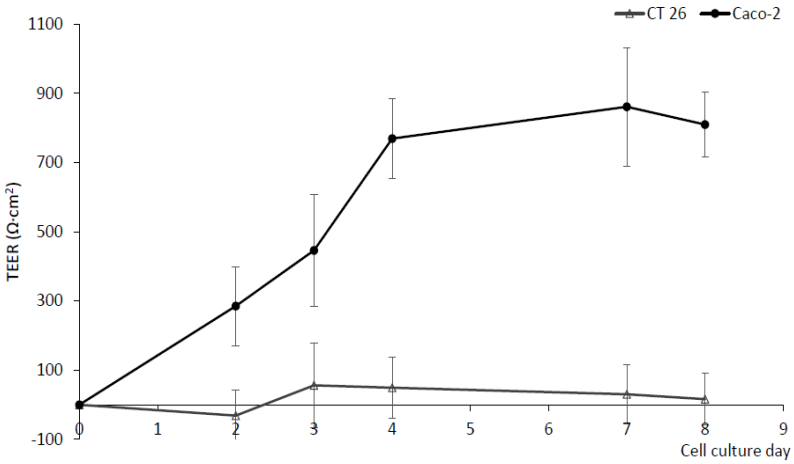

Figure 2. TEER measurements of Caco-2 cells and CT 26 cells. (n = 6, mean ± SD)

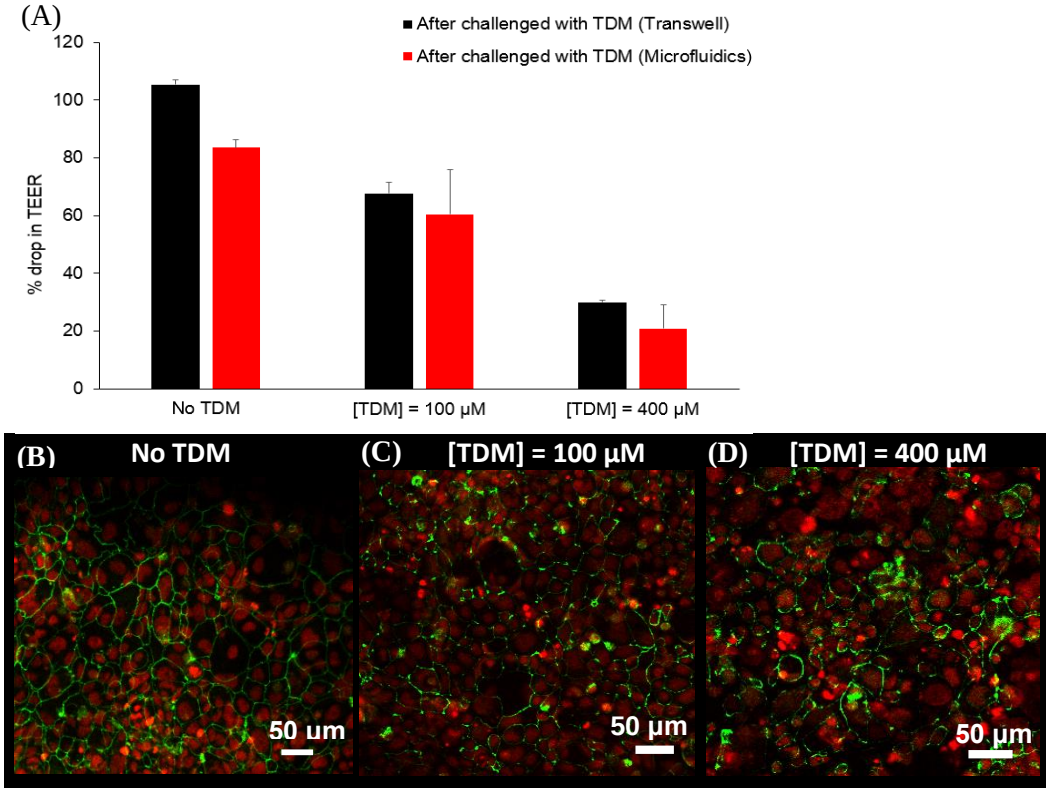

Figure 3. Impact of membrane enhancer, TDM, on the barrier integrity of Caco-2 monolayers. (A) Plot of TEER with relation to the presence or absence of TDM. Two different concentrations of TDM were investigated on the cell cultures in Transwell and microfluidic system. (n = 6). Immunostaining of Caco-2 monolayers for tight junctions, ZO-1 occuldens (green fluorescence) and nucleus (red fluorescence) when Caco-2 cells were subjected to: (B) no TDM; (C) [TDM] = 100 µM; (D) [TDM] = 400 µM. Cells were stained on day 9 of cell culture. Magnification at 10x.

CONCLUSION

We have successfully use a simple and straightforward method for fabricating microelectrodes in a multi-chamber microfluidic cell culture device for measuring cell barrier function. The capability of the microelectrodes in acquiring real-time measurements was demonstrated by measuring TEER across two different cell lines. Additionally, when the Caco-2 monolayers were challenged to different concentrations of a membrane enhancer, the microelectrodes showed capability in sensing dynamic changes to the barrier property. Immunofluorescence staining towards the tight junctions of the Caco-2 monolayers were also

conducted to further confirm the validity of the TEER measurements. This method of embedding the electrodes in the microchip could be carried out in ambient environment, eliminating any forms of using multiple lithography technologies, hence reducing labour fabrication costs. With such a set-up, had provided a solution to the limited existing equipment for acquiring TEER measurements in microfluidic devices for cell culture.

ACKNOWLEDGMENTS
This work is supported by The Danish Council for Independent Research (FTP) and the Lunbeck Foundation.

REFERENCES
1. Douville NJ, et al. (2010) Fabrication of two-layered channel system with embedded electrodes to measure resistance across epithelial and endothelial barriers. *Anal Chem* 82(6):2505–11.
2. Odijk M, et al. (2015) Measuring direct current trans-epithelial electrical resistance in organ-on-a-chip microsystems. *Lab Chip* 15(3):745–52.
3. Tan HY, et al. (2014) Multi-Chamber and Multi-Layer Thiol-Ene Microchip. *18th International Conference on Miniaturized Systems for Chemistry and Life Sciences* (San Antonio, Texas, USA), pp 1749–1751.